VECTORS AND ALTERNATIVE HOSTS OF TbYDV IN SOUTH-EASTERN AUSTRALIA

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Abstract

Factors that determine the epidemiology of *Tobacco yellow dwarf virus* (TbYDV), including alternative host-plants and insect vector(s), were assessed over three consecutive growing seasons at four field sites in Northeastern Victoria in commercial tobacco growing properties. In addition these factors were assessed for one growing season at three bean growing properties. Overall twenty-three leafhopper species were identified at the seven sites, with *Orosius orientalis* as the predominant leafhopper. Of the leafhoppers collected, only *O. orientalis* and *Anzygina zealandica* tested positive for TbYDV by PCR. The population dynamics of *O. orientalis* was assessed using sweep net sampling over three growing seasons and a trimodal distribution was observed. Despite large numbers of *O. orientalis* occurring early in the growing season (September-October), TbYDV was only detected in these leafhoppers between late November and the end of January. The peaks in the detection of TbYDV in *O. orientalis* correlated with the observation of disease symptoms in tobacco and bean and were associated with warmer temperatures and lower rainfall. Spatial and temporal distribution of vegetation at selected sites was determined using quadrat sampling. Of 40 plant species identified, TbYDV was detected in only four dicotyledonous species, *Amaranthus retroflexus*, *Phaseolus vulgaris*, *Nicotiana tabacum* and *Raphanus raphanistrum*. The proportion of host and non-host availability for leafhoppers was associated with climatic conditions.

Introduction

Tobacco yellow dwarf virus (TbYDV, family *Geminiviridae*, genus *Mastrevirus*) is an important pathogen in Australia causing summer death and yellow dwarf disease in bean (*Phaseolus vulgaris* L.) and tobacco (*Nicotiana tabacum* L.), respectively (Hill, 1941; Helson, 1950; Thomas & Bowyer, 1979; Thomas & Bowyer, 1980). Infection can result in annual losses of up to four million Australian dollars in tobacco (Trębicki *et al*. 2007) and symptoms of TbYDV infection and associated crop losses have been observed in bean crops in the Ovens Valley region (Trębicki pers. observ.)

TbYDV is a leafhopper-transmitted, phloem-restricted mastrevirus which is indigenous to Australia. Although most mastreviruses infect monocotyledonous plants, TbYDV together with *Bean yellow dwarf virus* from South Africa and *Chickpea chlorotic dwarf Pakistan virus* from Pakistan (Nahid *et al.* 2008), infect dicotyledonous plants. The complete genomic sequence of an Australian TbYDV isolate, derived from a tobacco plant growing in the Ovens Valley, Victoria, has been characterised and was shown to be monopartite and comprised of 2.58 kb of single-stranded, circular DNA (Morris *et al.* 1992).

The majority of studies investigating the epidemiology of TbYDV were conducted in the 1940's when specific and sensitive molecular diagnostic tests were unavailable and disease diagnosis was based on host-plant symptoms. Hill (1941) and later Helson (1950) reported that the leafhopper *Orosius orientalis* (Matsumura) (Hemiptera: Cicadellidae), previously described as *O. argentatus* (Evans) and *Thamnotettix argentata* (Evans), was a vector of the disease, but there has been little research aimed at identifying other potential vectors. Furthermore,

although numerous host-plants for TbYDV have been reported (Helson, 1950; Hill, 1950; Hill & Mandryk, 1954; Bowyer & Atherton, 1971), most of these have been identified under experimental conditions by the development of typical yellow dwarf symptoms in tobacco (Hill, 1937) following transmission from suspected host-plants by either grafting or by the vector *O. orientalis*. Recently, a TbYDV-specific PCR test was developed (van Rijswijk *et al.* 2004) which was used to determine the variability within the genome of Australian TbYDV isolates. This PCR test was also used to identify the weed *Raphanus raphanistrum* as a host-plant of TbYDV (van Rijswijk *et al*. 2004).

Due to the lack of detailed epidemiological data for TbYDV, disease management strategies continue to rely heavily on repeated insecticide applications which are largely ineffective and environmentally hazardous (Paddick *et al.* 1971; Paddick & French 1972; Osmelak 1986). The aim of this 3-year study was to use the molecular-based diagnostic test developed by van Rijswijk *et al.* (2004) to conduct a more thorough investigation into the epidemiology of TbYDV through seasonal monitoring of plants and Hemipteran insects in vegetative areas surrounding bean and tobacco crops at commercial farms in south-eastern Australia. Such data should assist in the development of improved management strategies to control diseases caused by TbYDV in commercial crops.

Materials and methods

Sampling sites

The collection of potential leafhopper vectors and monitoring of their population dynamics was conducted over three consecutive growing seasons, 2005/06,

2006/07 and 2007/08, at four commercial tobacco (*Nicotiana tabacum* L.) farms located in the Ovens Valley, north-east Victoria, Australia (Figure 3.1). These farms have been under continuous tobacco cultivation using conventional management conditions for decades. Sites A and B were located in the Lower Ovens Valley near Whorouly (36°29'23S, 146°35'48E) with an elevation of 210 metres above sea level (masl). Site C was located between Ovens and Eurobin (36°37'12S, 146°48'31E) at 246 masl while Site D was located in the Upper Ovens Valley (36°43'25S, 146°52'30E) with an elevation of 320 masl. During the 2007/08 growing season, sampling was also carried out at an additional three sites (E, F and G) near Myrtleford (36°33'S, 146°34'E) where tobacco had been previously grown but which had been subsequently replanted to beans (*Phaseolus vulgaris* L. cv. "Borlotti").

Site selection was based on the historical incidence of TbYDV symptoms in commercially grown tobacco. Historically, tobacco growing in the Lower Ovens Valley region (sites A and B) is most affected by TbYDV, with decreasing disease incidence recorded with increasing elevation (sites C and D) (G. Baxter personal observation).

Weather data

Meteorological data for each collecting season was obtained from the SILO database (http://www.bom.gov.au/silo/). Air temperature and rainfall were recorded from three weather stations each located within five km of each experimental site.

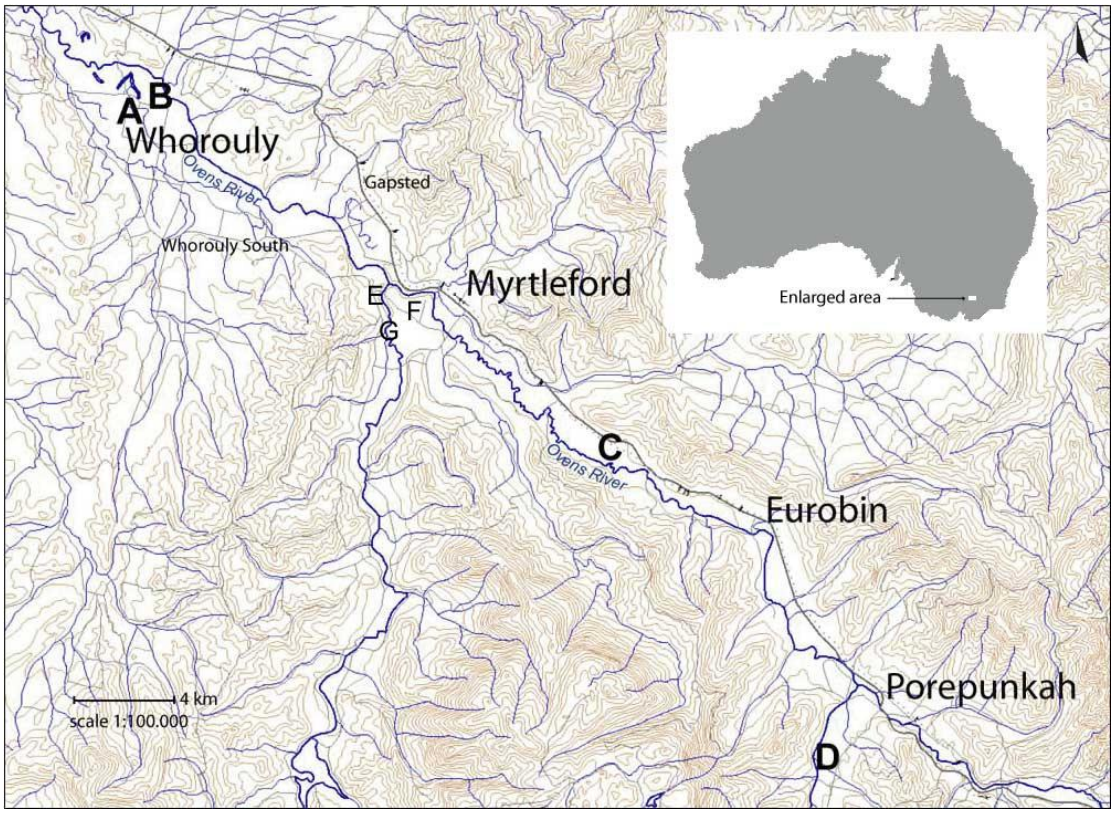

Figure 3.1 Location of field sites (labelled A – G) in north-east Victoria, Australia, that were used in this study.

Leafhopper collection

During each season, leafhoppers were collected on a weekly basis using a sweep net (38 cm diameter). Sweeping was done by swinging the net through the plant canopy at 180° along a 100 m transect where one metre was equivalent to one sweep (Figure 3.2). One hundred sweeps per site per sampling date were done during the 2005/06 and 2006/07 seasons, while 150 sweeps per site (sites A and B) per sampling date were done during the third season. In addition, leafhoppers were collected weekly at sites E, F and G between December 2007 and January 2008. The insects caught were immediately placed in a killing jar containing ethyl acetate for 10-15 mins before transfer into zip-lock plastic bags. To sort samples, four different laboratory test sieves were stacked on top of each other in descending pore size (aperture size: 3.35 mm; 2 mm; 1 mm and 212 µm). Samples were emptied onto the sieves and briefly shaken. Each sieve was emptied and all leafhoppers were transferred to a Petri dish for microscopic identification using taxonomic keys (Evans, 1966; Ghauri, 1966; Knight, 1975; Knight, 1987; Day & Fletcher, 1994; Fletcher, 2009 and updates). Identified specimens were transferred to a 1.5 ml test tube and stored at -80°C for DNA analysis.

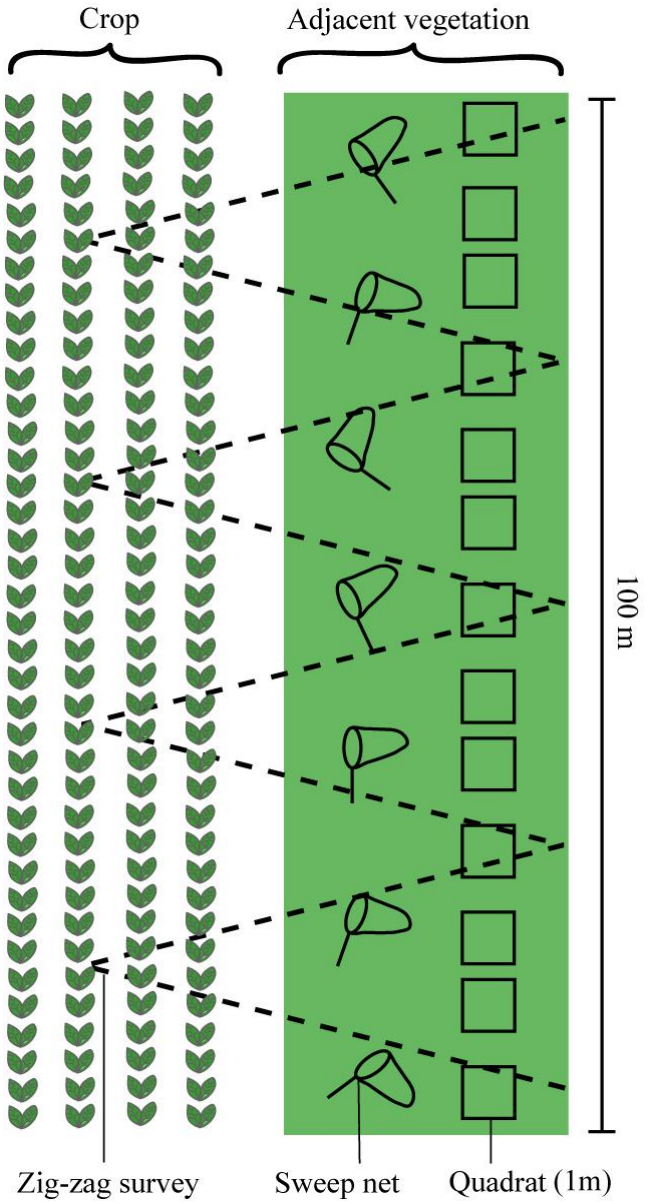

Figure 3.2 Overview of the sampling methodology used for insects (sweep net) and plants (quadrant) at each field site.

Plant surveys

To determine the potential host-plants for TbYDV, a diverse range of plant species were collected at monthly intervals at four experimental sites (A, B, C and D for the first two seasons; A and B only in the third season). In addition, plant material of selected species (namely *Phaseolus vulgaris*, *Nicotiana tabacum*, *Amaranthus retroflexus* and *Raphanus raphanistrum*) was collected at three sites (E, F and G), on five occasions between December 2007 and January 2008. A line transect sampling method using a zigzag survey design (Strindberg & Buckland, 2004) was utilized to collect all plant material. The zig-zag survey was done both within crop and in adjacent vegetation along a 100 m transect at every site (Figure 3.2). Randomly selected specimens of each plant species located on the transect line were collected and identified to species level using field guides and classification keys (Lamp & Collet 1989; Parsons & Cuthbertson 1992; Cummins & Moerkerk 1997). Samples were placed in plastic zip-lock bags and stored at either 4°C or -80°C for further analysis using PCR to determine TbYDV presence.

The quadrat survey method was used to assess the presence of potential host-plants for leafhopper vectors identified in the previous growing season (2005/06), to understand differences between the sites and to collect information regarding changes in plant temporal composition and predominant ground cover. Leafhopper host-plant surveys were carried out at monthly intervals at four locations (A-D) during one growing season only (December 2006-April 2007). Using a 1 m^2 metal quadrat, 29 measurements along 100 m long plots were taken at each sampling date from each site (Figure 3.2). To ensure the quadrat was placed in the same sampling location at each sampling date, a grid of wooden stakes were placed permanently on-site during the season at each location. For each quadrat, the plant species present, the percentage of coverage of each plant

species and dry plant coverage was visually assessed. All plants were identified to species level using field guides (Lamp & Collet, 1989; Parsons & Cuthbertson, 1992; Cummins & Moerkerk, 1997).

In addition to the quadrat survey in areas adjacent to the tobacco crop, during 2006 and 2007 vegetation at all seven field sites was visually assessed for symptoms of TbYDV and random symptomatic (i.e. showing yellowing and stunting) plant samples were collected and tested for presence of the virus by PCR.

DNA extraction and PCR

DNA was extracted from leafhoppers essentially as described by Goodwin *et al*. (1994). Depending on the number of leafhoppers collected at each sampling point between 1-10 leafhoppers of a single species were pooled and placed in 1.5 ml Eppendorf tubes containing between 125 µl to 500 µl CTAB extraction buffer, crushed using a micropestle and incubated at 65°C for 5 min. Following a chloroform/isoamyl alcohol (24:1 v/v) extraction, the DNA was precipitated, washed twice in 70% ethanol and resuspended in nuclease-free sterile water. Samples were stored at -20°C. DNA was extracted from plants (~0.2 g of leaf midrib/petiole tissue) using a QIAGEN DNeasy® Plant Mini kit according to the protocol described by Green *et al.* (1999). The samples were resuspended in 100 µl TE buffer, pH 9.0 and stored at -20°C.

Both insect and plant sample DNA was tested for the presence of TbYDV using the TbYDV-specific primers, TYDVF (5’ CAT TTA TAT TGG TAG GTG GAC 3’) and TYDVR (5’CCC TTA TAC CGG CCC GCC AT3’), which amplify a 509 bp product (van Rijswijk *et al.* 2004). Each PCR reaction (25 µl) contained 0.5 µl of DNA template, 1 µl of each primer (0.4 µmol final concentration) and

either 22.5 µl of Invitrogen™ Platinum® PCR SuperMix or, a reaction master mix containing 18.75 µl of sterile (RNase-, DNase-free) water, 2.5 µl 10 x reaction buffer, 0.75 µl 50 mM $MgCl_2$, 0.5 µl dNTP mixture (each at 10 mM). The mixture was heated at 95°C for 10 min and subjected to 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min and a final step of 72°C for 10 min using a BioRad Icycler. Amplicons were analysed by electrophoresis through 1% TBE agarose gels and visualised by ethidium bromide staining. Three controls were included in all gels (known TbYDV infected tobacco, non-infected tobacco and no template).

Statistical analysis

Statistical analysis was performed using Genstat software (10th Edn© 2007, Lawes Agricultural Trust). A one-way analysis of variance (ANOVA) was used to compare differences between four sites (A-D) in relation to annual temperature during three years (2005-07).

Results

Leafhopper diversity and abundance

Using a sweep net, in excess of 4300 leafhoppers were collected at the seven field sites over the three-season survey period. These leafhoppers were classified into 23 species (Table 3.1), of which two were previously undescribed. *Orosius orientalis* was by far the most abundant species (60%) found across all sites followed by *Anzygina zealandica* (Myers) (11%), previously known as *Zygina zealandica* (Fletcher & Larivière 2009), and *Nesoclutha phryne* (Kirkaldy) (10%).

Relatively high numbers of *Balclutha frontalis* (Ferrari) (6%), *Austroasca viridigrisea* (Paoli) (5%), *Batracomorphus angustatus* (Osborn) (2%) and *Arawa novella* (Metcalf) (2%) were also collected. During the first two growing seasons, most *O. orientalis* were collected from site C (n = 1474), followed by site B (n = 625), site D (n = 422) and site A (n = 255). During the third growing season, when insects were only collected at sites A and B, the number of *O. orientalis* was again higher at site B (n = 452) than site A (n = 290).

Testing leafhoppers for TbYDV

Of the 23 leafhopper species tested for the presence of TbYDV by PCR, a product of the expected size (~500 bp) was only amplified from *O. orientalis* and *A. zealandica* samples. Of the 313 *O. orientalis* samples (each containing between 1-10 leafhoppers depending on the number collected during each sampling date) tested over the three season surveys, 70 samples (22%) were TbYDV positive (Table 3.2). Of these 70 samples, those derived from site B contained the greatest number of positives (32%) followed by site A (24%) while 10% of samples collected at sites C and D tested positive. In addition, all *O. orientalis* samples were positive at sites E-G

Of the 83 samples containing *A. zealandica* that were collected, only one sample from site B, which comprised a single insect, tested positive for TbYDV.

Table 3.1 Leafhoppers collected from seven field sites (A-G) during the three survey seasons from 2005-2008.

Subfamily	Species	Common name	Number collected
Agalliinae	*Austroagallia torrida* Evans	Spotted leafhopper	41
Deltocephalinae	*Arawa novella* Metcalf		92
	Chiasmus varicolor Kirkaldy		4
	Exitianus plebeius Kirkaldy		21
	Exitianus nanus Distant		1
	Limotettix incertus Evans		21
	Nesoclutha phryne Kirkaldy	Australian grass leafhopper	437
	Orosius orientalis Matsumura	Common brown leafhopper	2579
	Orosius canberrensis Evans		8
	Maiestas knighti Webb & Viraktamath		10
	Maiestas vetus Knight		4
Eurymelinae	*Balclutha frontalis* Ferrari		249
Iassinae	*Eurymeloides pulchra* Signoret		2
Typhlocybinae	*Batracomorphus angustatus* Osborn	Green jassid	95
	Austroasca viridigrisea Paoli	Vegetable leafhopper	222
	Kahaono pallida Evans		8
	Kahaono wallacei Evans		8
	Anzygina zealandica Myers	Yellow leafhopper	463
	Anzygina sidnica Kirkaldy		20
	Zygina evansi Ross		16
Xestocephalinae	*Xestocephalus australensis* Kirkaldy		15

Table 3.2 Prevalence of TbYDV in samples of *Orosius orientalis* collected from different field sites during 2005-2008.

	Site	Samples tested[a]	TbYDV positive samples
O. orientalis	A	37	9 (24%)
	B	148	48 (32%)
	C	98	10 (10%)
	D	30	3 (10%)

[a] = each sample contained 1-10 leafhoppers.
Additionally *Orosius orientalis* was collected on a few occasions from sites E, F and G and all samples tested positive for TbYDV

Population dynamics of *O. orientalis*

When the number of *O. orientalis* trapped at the four sites (A-D) in consecutive growing seasons was analysed, a trimodal peak was generally observed (Figure 3.3). At all sites, similar trends were observed in the seasonal activity of *O. orientalis*. The first population peaks occurred early in the season mid-September (early spring), the second in late November and the third in early January (summer). At sites C and D, the greatest numbers of *O. orientalis* were recorded in spring, whereas at sites A and B, peak numbers were recorded during the summer. The numbers of *O. orientalis* remained at relatively low levels from the end of January until late May. When the relative abundance of *O. orientalis* between sites was analysed, relatively higher numbers of leafhoppers were observed in the second survey season, a season characterised by relatively low annual rainfall and warmer temperatures (see below).

Potential alternative hosts of TbYDV

A diverse range of weeds and cultivated plant species growing adjacent to commercially grown tobacco and beans were collected using a zig-zag survey and tested for the presence of TbYDV by PCR (Table 3.3). Of the 40 plant species tested, TbYDV was only detected from samples of *Phaseolus vulgaris* (common bean), *Nicotiana tabacum* (tobacco) and the weeds *Amaranthus retroflexus* L. and *Raphanus raphanistrum* L. Although both *A. retroflexus* L. and *R. raphanistrum* were identified at all sites, TbYDV-infected samples of these plant species were only detected at sites A-C and E-G. When pooled samples of each known TbYDV host-plant species (shown in Table 3), collected monthly from zig-zag surveys over the three year period, were tested using PCR, the greatest number of

TbYDV-positive samples was found at site B (46%; 14 positives/30 samples), followed by site A (43%; 13/30), site C (13%; 4/30) and site D (3%; 1/30).

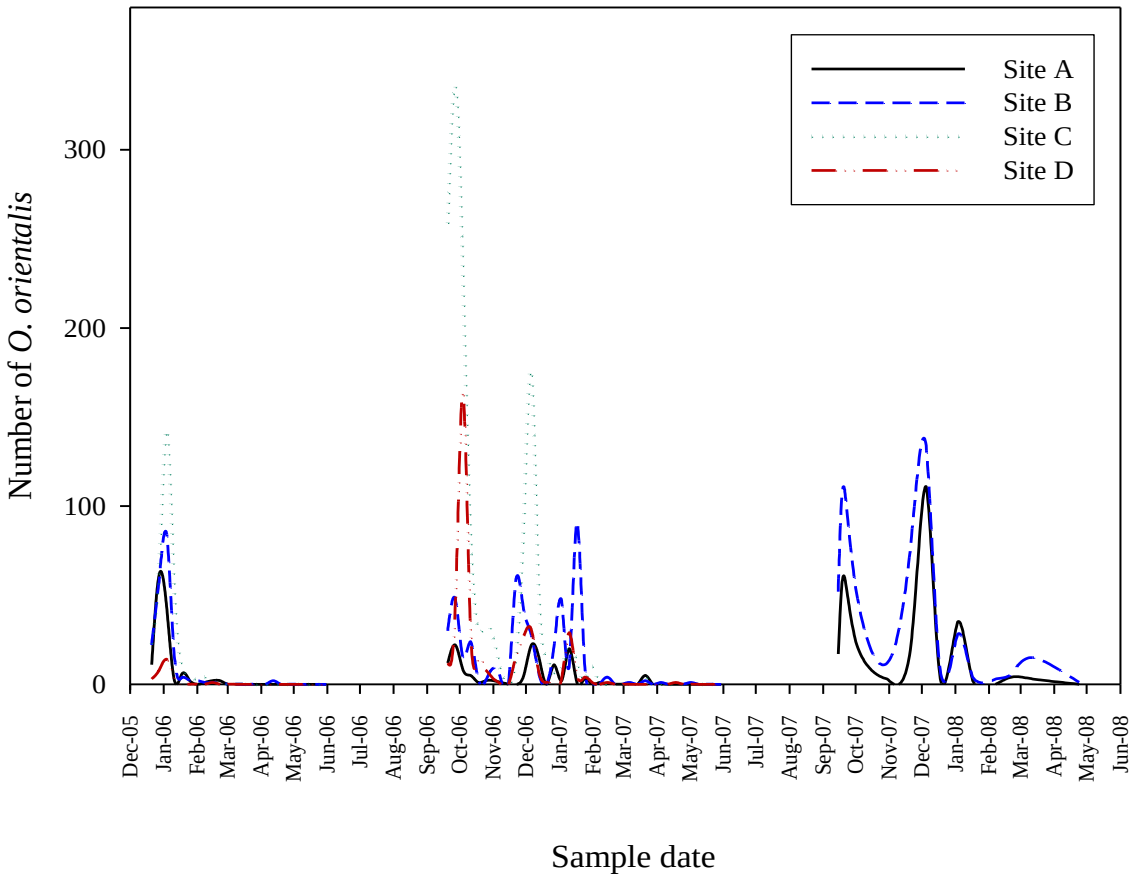

Figure 3.3 Population dynamics of *Orosius orientalis* during three growing seasons (2005/06, 2006/07 and 2007/08) from four sites (A, B, C and D) monitored using sweep netting. In the 2007-08 collecting season only sites A and B were studied.

Table 3.3 Average minimum and maximum temperature and total annual rainfall recorded at field sites A-D from over three years (2005-07)

Year	2005			2006			2007		
Sites	Temp. (°C)		Rain (mm)	Temp. (°C)		Rain (mm)	Temp. (°C)		Rain (mm)
	max	min		max.	min.		max.	min.	
A & B	21.71	7.63	815.4	23.15	7.1	333.4	22.98	8.88	653.7
C	21.32	7.82	1229.8	22.66	7.44	449.4	22.52	9.07	954.8
D	18.92	6.72	1196.8	20.22	6.47	476.8	20.04	7.86	863.5

Table 3.4 Plant species tested for presence of TbYDV obtained from four field sites (A, B, C and D) collected during three growing seasons

Plant species	TbYDV-status of plants from different sites**				Plant species	TbYDV-status of plants from different sites**			
	A	B	C	D		A	B	C	D
Acacia spp.	×	-	-	-	*Plantago lanceolata*	-	×	-	×
Amaranthus retroflexus	+	+	+	+	*Plantago major*	-	-	-	×
Bromus diandrus	-	-	-	-	*Polygonum aviculare*	×	-	-	×
Bromus mollis	-	-	×	-	*Polygonum hydropiper*	×	×	-	×
Capsella bursa-pastoris	×	-	-	×	*Raphanus raphanistrum*	+	+	+	-
Chenopodium album	-	-	-	×	*Rubus fruticosus*	×	-	-	×
Citrullus lanatus	×	-	×	×	*Rumex acetosella*	×	×	×	-
Convolvulus arvensis	×		-	×	*Rumex crispus*	×	×	-	×
Cynodon dactylon	-	-	×	-	*Salvia verbenaca*	×	-	-	×
Cyperus egrostis	×	-	-	×	*Silybum marianum*	×	-	-	×
Datura spp.*	-	-	-	×	*Solanum lycopersicum*	-	-	×	×
Galium aparine	×	×	-	×	*Solanum nigrum**	×	-	×	×
Humulus spp.	×	×	-	×	*Stachys arvensis*	×	×	-	×
Lactuca serriola	-	×	-	×	*Taraxacum officinale*	-	-	-	-
Lolium rigidum	-	-	-	-	*Trifolium repens**	-	-	-	-
*Malva parviflora**	-	-	-	×	*Trifolium subterraneum**	×	×	-	-
Nicotiana tabacum	+	+	+	+	*Typha domingensis*	×	×	-	×
Olea europaea	×	-	×	×	*Vicia sativa*	×	×	-	×
Paspalum spp.	-	-	-	-	*Vitis* spp.	-	-	×	×
Phaseolus vulgaris	+	+	×	×	*Zea mays*	-	×	×	×

+ = PCR positive, - = PCR negative, × = plants not present at the site. *confirmed hosts plants for *O. orientalis.* ***Phaseolus vulgaris, Raphanus raphanistrum* and *Amaranthus retroflexus* also tested positive at sites E-

Using the quadrat survey, the temporal distribution and diversity of vegetation from four sites (A-D) was monitored through one season (December 2006 to April 2007) in order to assess the differences between the sites in terms of plant composition and distribution and occurrence of host-plants for the vector and the disease. Forty-two plant species were identified during this period (data not presented). Site C had the highest number of different plant species followed by sites B, A and D, respectively (Figure 3.4). Additionally, site C had the greatest diversity and density of known *O. orientalis* host-plant species followed by B, A and D. With the exception of site D, where the highest proportion of plant species recorded were monocotyledonous, all other sites were dominated by dicotyledonous plants. Even though monocots were represented by fewer species (sites A, B and C), they had the highest spatial density in all sites. At each site, *Cynodon dactylon* L. was the dominant plant species with between 15% (site B) to 40% (sites A, C and D) coverage at the beginning of the season. At sites A, B and C, *Raphanus raphanistrum*, a known host-plant for TbYDV, was found at 1-5 % coverage. In contrast, only single plants of another known host-plant, *A. retroflexus,* were recorded from all sites during the quadrat survey period. The virus status of these plants was not examined. During the survey period (December 2006 – April 2007), the neighbouring farms at sites A and B had the same total rainfall (123 mm each) which was almost two-fold lower than that received at sites C (216 mm) and D (201 mm). Despite identical rainfall at sites A and B, the proportion of senesced vegetation, dicotyledonous and monocotyledonous species at each site throughout the season was different with monocots more prevalent at site A and dicots more prevalent at site C (Figure 3.5). There was a similar trend between changes in the proportion of dry vegetation during the sampling period between sites A and D.

Seasonal detection of TbYDV

Leafhoppers and plants were collected from September till the end of May over the three survey seasons on a weekly and monthly basis at four sites (A-D), respectively, and tested for the presence of TbYDV. In addition at sites E-G samples were only collected and tested once during January 2008.

Monitoring conducted on Cicadellidae trapped at each site indicated that, although a diverse range of leafhopper species (over 23 species) were prevalent in and around each of the commercial tobacco and bean farms, only *O. orientalis* was consistently trapped in high numbers in all seasons, despite relatively high numbers of leafhoppers and host-plants early in each season (during spring). No TbYDV-positive samples were detected until the end of November. TbYDV was subsequently detected from the end of November until February in *O. orientalis* (Figure 3.6) and four plant species (*P. vulgaris*, *N. tabacum*, *A. retroflexus* and *R. raphanistrum;* Table 3.3). The characteristic symptoms of TbYDV were observed in both beans and tobacco between six to ten days after the *O. orientalis* population peak in early January 2006/07 for tobacco and early January 2008 for beans. In mid-January 2006 in a tobacco field adjacent to sites A and B around 30% of the crop was infected with TbYDV compared to site C where TbYDV infection was around 5% and site D with only a few infected tobacco plants detected around the field border. This survey was conducted based on a visual assessment of typical symptoms of TbYDV in tobacco (Hill, 1937) and confirmed by PCR. Additionally, in January 2008, up to 95% of the *P. vulgaris* samples collected at three bean farms (E, F and G) in Myrtleford tested positive for TbYDV by PCR and total crop failure was observed.

Meteorological data collected over the three-season survey period (2005-07) showed that the average annual maximum temperatures at sites A, B and C were significantly higher ($P<0.05$) than that of site D (Table 3.4). Rainfall data showed that site C recorded the highest

precipitation rate (apart from 2006) followed by site D and with the lowest recorded for sites A and B.

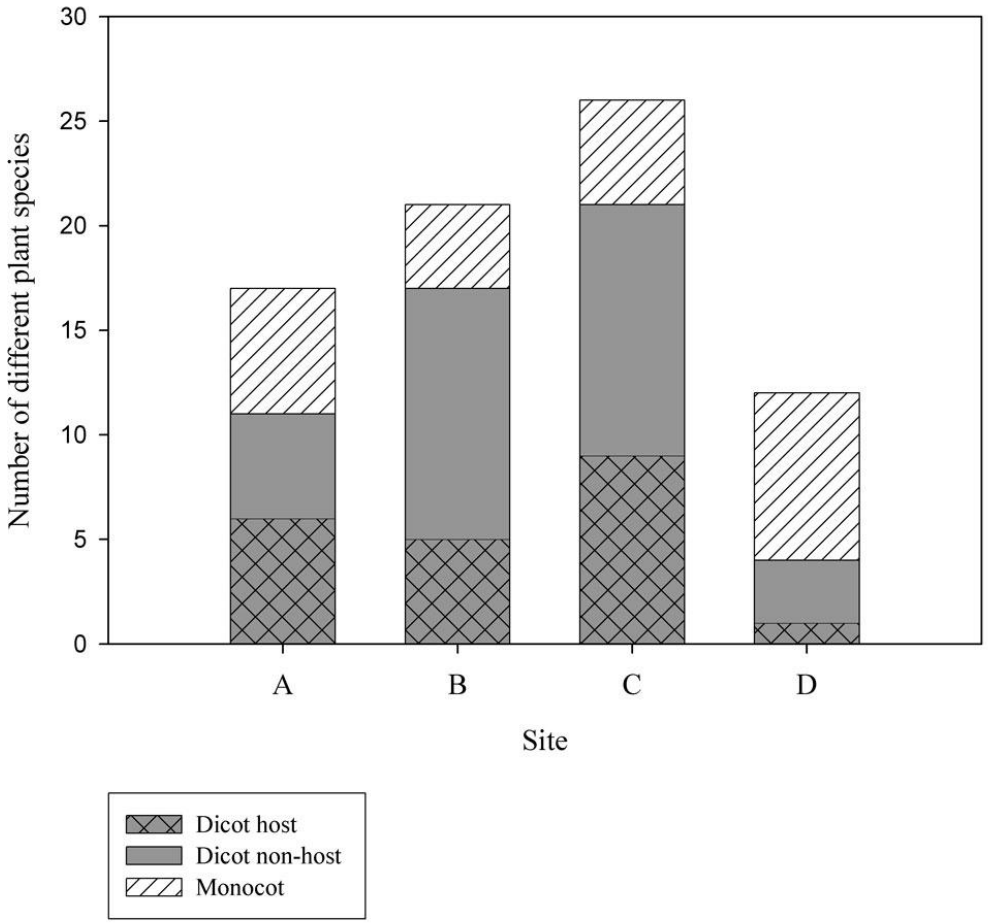

Figure 3.4 Proportion of total host and non-host-plants for *Orosius orientalis* from four field sites (A-D) recorded during 2006/07 growing season as determined by quadrat survey.

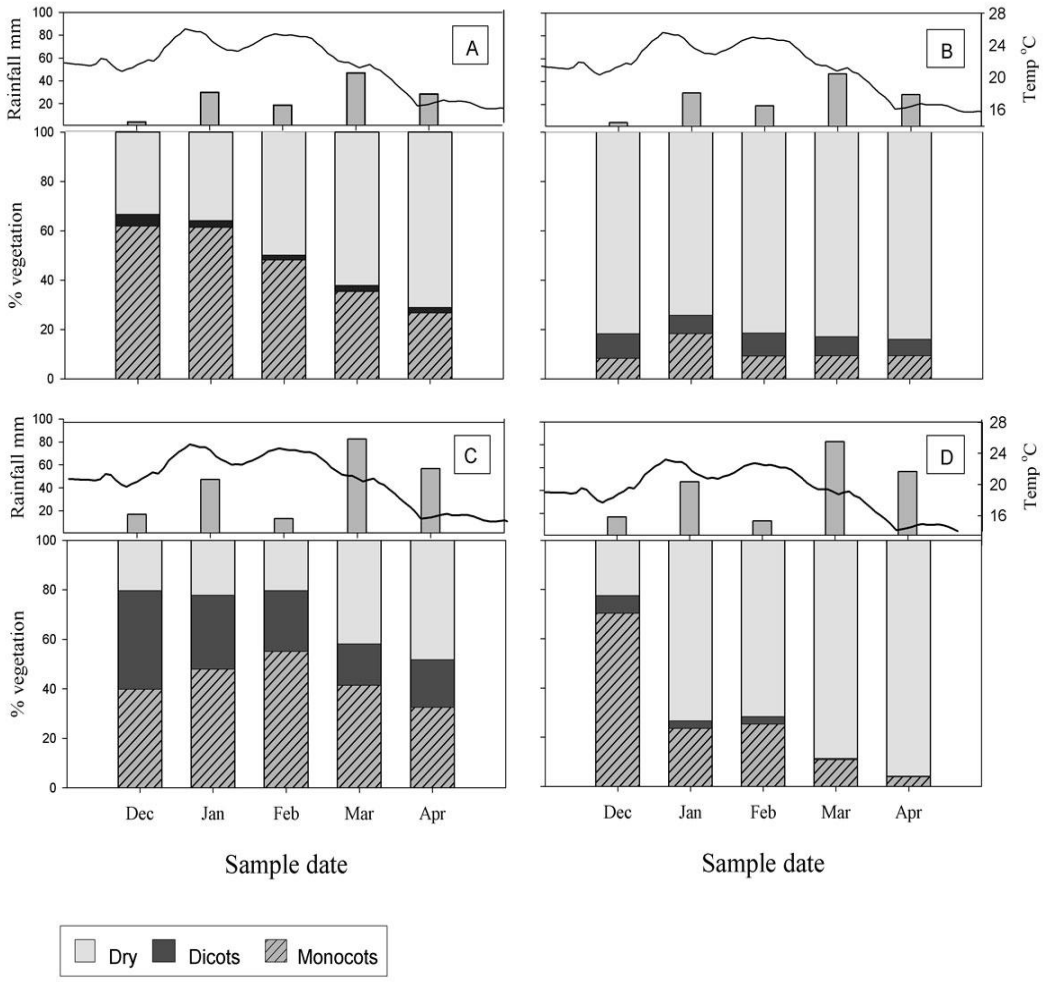

Figure 3.5 Changes in distribution of vegetation type [senesced (dry), dicotyledonous and monocotyledonous], rainfall and temperature during one growing season (2006/2007) across the four field sites (A, B, C and D, lines = average temperature, bars = rainfall).

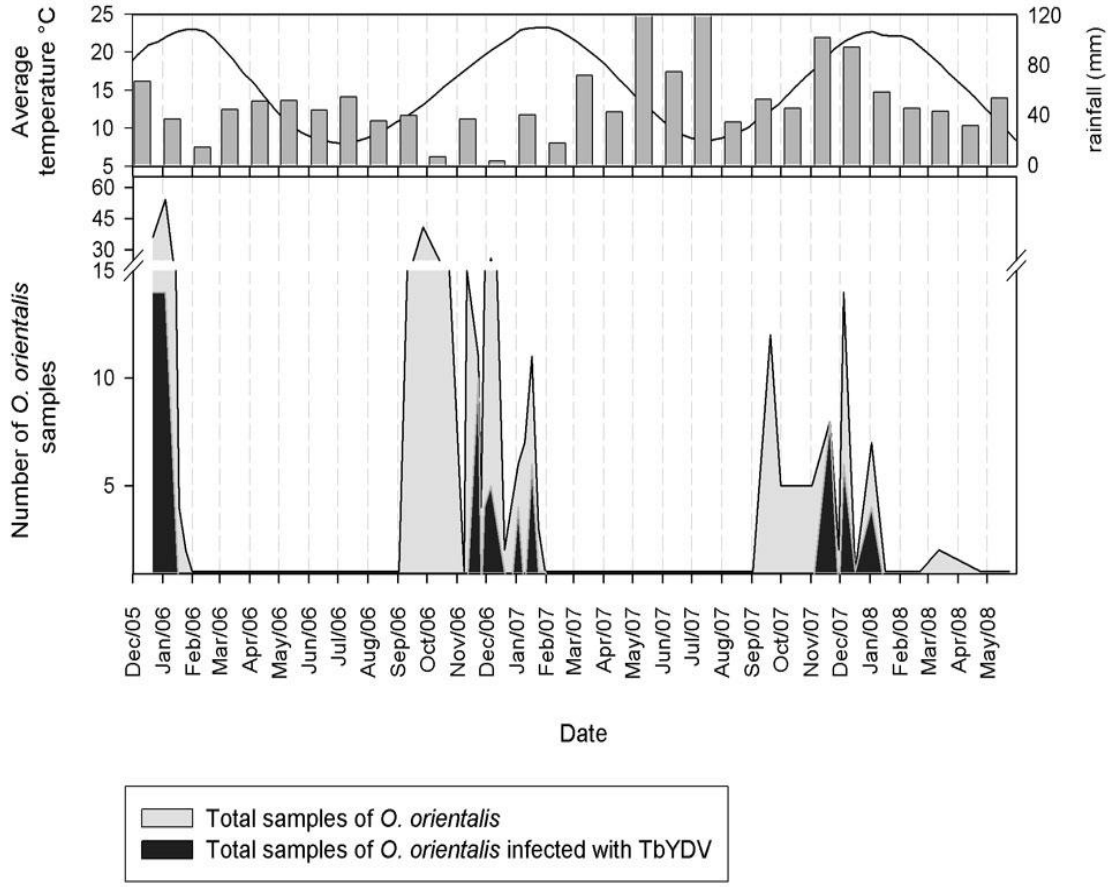

Figure 3.6 Numbers and TbYDV status of *Orosius orientalis*, average temperature and rainfall during three consecutive growing seasons. Combined data from all four field sites: A, B, C and D (lines = average temperature, bars = rainfall).

Discussion

This is the first systematic epidemiological study of TbYDV achieved by examining the interactions between the virus, leafhopper vector/s and host-plants under field conditions. Hill (1941) first reported that *O. orientalis* was a possible vector of TbYDV based on the development of typical disease symptoms on healthy tobacco that had been exposed to a range of field-collected leafhoppers. In a previous study using a PCR-based diagnostic test (van Rijswijk *et al.* 2002), TbYDV was detected not only in *Orosius orientalis*, but also in the leafhoppers *Balclutha* spp., *Limotettix incertus* and *Anzygina* spp. In the present study up to 32% of the *O. orientalis* samples tested positive for TbYDV by PCR, although the relative percentage may be lower (due to sample size variation) this still provides some evidence that this insect is an important vector. This study did not detect TbYDV in *Balclutha* spp or *L. incertus,* despite regular testing over three seasons, and only one sample of 83 *A. zealandica* tested positive for TbYDV. Therefore it is unlikely that *Balclutha* spp is a vector of TbYDV as it feeds predominately on grasses (Knight 1987; Pilkington *et al.* 2004; Narhardiyati & Bailey 2005) and TbYDV has only been detected in dicots. Furthermore, there is no evidence that this species is a phloem feeder. In contrast *L. incertus* is predominantly a phloem feeder and could theoretically acquire the phloem-specific TbYDV (Day *et al.* 1952). However, the relatively small numbers of this leafhopper trapped in the field suggest that *L. incertus* is unlikely to be an important vector, at least in the area studied. Although TbYDV was detected in *Anzygina* spp, both in this study and that of van Rijswijk *et al*. (2002), this leafhopper is also considered an unlikely vector as both nymphs and adults feed preferentially on parenchymal cells (Witt & Edwards 2000) rather than phloem (Day *et al.* 1952).

However transmission tests, using known host-plants, will ultimately be required to prove the vector status of these insects.

Orosius orientalis is widely distributed in Australia, Asia and the Pacific region (Ghauri 1966). It is considered a polyphagous insect, having been recorded as feeding on over 60 plant host species, including *A. retroflexus* and *R. raphanistrum*, and confirmed to be able to breed on many of these hosts. The detection of TbYDV-infected *A. retroflexus* and *R. raphanistrum* in this study supports the notion that these weeds may act as reservoirs for virus acquisition by *O. orientalis*. However, Tobacco is not considered to act as a virus reservoir since (i) no nymphal development and only limited adult survival has been recorded on *N. tabacum* (Hill, 1941; Helson, 1942) and (ii) TbYDV transmission and acquisition studies have shown that *O. orientalis* cannot acquire the virus from TbYDV-infected tobacco (Helson, 1950). As such, tobacco should be considered a dead-end host for the virus as this plant species only becomes infected when the preferred leafhopper hosts decline and the insect is forced to feed on it. In contrast, *P. vulgaris* can support the insect through at least one generation and can therefore potentially serve as a virus reservoir (Bowyer & Atherton, 1971).

The population dynamics of *O. orientalis* showed three peaks of abundance across four sites (A-D) occurring during the three survey seasons (during two tobacco growing seasons (from November-April) and one bean growing season at three sites (E-G; from December-March)). Typical symptoms of TbYDV were clearly visible in both tobacco and bean crops between six to ten days after the population peak in early January, with virus infection confirmed by PCR. This is consistent with previous studies which reported the occurrence of disease symptoms in late November, late December and early January (Hill & Allan, 1942; Hill, 1950). Interestingly, although in the present study TbYDV was detected in *O. orientalis* in late November, no TbYDV was detected in crop plants until January. It is

possible that temperature may have a major influence on the ability of vectors to acquire and transmit TbYDV. In our study, TbYDV was detected in both insect and plants only when average temperatures were above 18°C. Research with other Cicadellid vectors has also shown that virus transmission is greatly reduced or abolished at lower temperatures (Hitoshi & Jutaro, 1980; Creamer & He 1997).

A combination of many factors, including climate and vegetation, may play a major role in the incidence of TbYDV across the Ovens Valley. Clear differences were observed between sites in regards to the occurrence of disease, weed hosts and *O. orientalis*. Plant composition and leafhopper diversity differed between sites, especially between sites located in the Lower Valley, where the occurrence of TbYDV was highest (A and B) and sites in Upper Valley (C and D) where TbYDV was recorded relatively rarely. Sites A and B, which recorded the highest incidence of TbYDV in host-plants and *O. orientalis*, had the lowest precipitation and the highest average temperature. Although tobacco is not a preferred host for *O. orientalis*, reduced primary host-plant availability at these sites, especially in mid-summer, may have encouraged the vector to feed on the irrigated tobacco crop when border vegetation had dried out. At sites C and D, which had relatively lower maximum temperature (site D) and higher rainfall than sites A and B the virus incidence was lower than that recorded from sites A and B. At site C, although host-plant species for *O. orientalis* were the most abundant, the incidence of TbYDV-infected host-plants bordering the field site was considerably lower than that at sites A and B. Even though the vector was in high abundance at site C, higher rainfall and less host-plants for the virus reduced the incidence of the disease. Site D was less affected by the virus because it was surrounded mainly by grazing land with a higher proportion of non-host monocotyledonous species, a very low number of suitable host-plant species for the vector and the virus, as well as a relatively high rainfall and lower temperature. Incidence of TbYDV in the vector and the host-plant appeared at the same time

at each location, despite the site differences in terms of vector numbers and host-plant diversity.

The results of this study have provided important information which can be used to develop more effective disease control strategies. For example, the population dynamic studies have provided data on the seasonal occurrence of *O. orientalis* and thus the most appropriate times for insecticide applications. Furthermore, disease occurrence in beans and tobacco could be minimised by targeting herbicide applications to TbYDV host-plants such as *Amaranthus retroflexus* and *Raphanus raphanistrum*) that were commonly found in and around crops at the two most disease-affected sites. Apart from TbYDV, *O. orientalis* also transmits phytoplasmas which cause a range of economically important phytoplasma-associated diseases in Australia such as legume little leaf (Hutton & Grylls, 1956), tomato big bud (Hill & Mandryk, 1954; Osmelak, 1986), lucerne witches broom (Helson, 1951), potato purple top wilt (Grylls, 1979; Harding & Teakle, 1985) and Australian lucerne yellows (Pilkington *et al*. 2004). Therefore, the results from this study will possibly lead to effective control strategies for not only TbYDV, but also for phytoplasma diseases vectored by *O. orientalis*.

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